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FOR THE STUDY OF

THE [illegible]OLOGICAL ACTION OF DRUGS

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LABORATORY OF

PHYSIOLOGY AND PHARMACOLOGY

UNIVERSITY OF MISSOURI

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EXPERIMENTAL PHARMACOLOGY

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THE ACTION OF DRUGS

OPERATIONS, APPARATUS AND SPECIAL DIRECTIONS

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NOTE.

The instruction in Pharmacology in the University of Missouri from the date of its establishment in the fall of 1900 has been based on a rigid course of required laboratory experiments. The student in Pharmacology no less than in Physiology should be given every opportunity to observe for himself the changes produced by a drug in the activities of a tissue, of an organ, and in the entire organism. It is only on such intimate personal experience with the facts that one can base a rational discussion of the principles of Pharmacology. The directions presented here have been formulated during the growth of the course as presented in this University and are now printed for the first time. Valuable aid and suggestion have been rendered by my coworkers, Dr. Waldemar Koch, and Mr. Omar Ray Gullion, now of Cornell University.

Charles W. Greene.

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THE ACTION OF DRUGS.

ALCOHOL.

The effects of alcohol.

1. **On the frog.**
2. **On the ventricular muscle.**
3. **On the frog's heart.**
4. **On muscle work.**
5. **On voluntary work of human muscle. Demonstration.**
6. **On the circulatory and respiratory systems of the mammal.**
7. **On the reaction time of the reflex frog.**

1. **Alcohol on the frog.** Inject into the dorsal lymph sacs of two frogs doses of **0.3cc** (5 minims) and **0.6cc** respectively of **95 percent alcohol.** Strong alcohol is quickly removed from the lymph sac. The larger dose above is sufficient to produce complete loss of reflexes including all respiratory movements. A dose of **1cc** is toxic for a 40 gram frog. Since the smaller dose above is equivalent to 525cc for a 70 kilo man, it is evident that the frog is the more tolerant of alcohol.

2. **Alcohol on the heart muscle.** Mount a strip of the ventricle of a terrapin in 0.7 percent sodium chloride and when it is contracting with an even and regular rhythm change to a solution of **2 percent alcohol** in physiological saline. Renew the saline after two to five minutes. Record the contractions on a drum moving 1 mm a second. Repeat with strengths of alcohol of **5 and 10 percent.** The alcohol effect will be demonstrated rather better on a ventricular strip that is contracting in the weaker Ringer's solution, page 45, but the alcohol must be dissolved in the same physiological solution.

3. **Alcohol on the frog's heart.** Destroy the brain and cord ~~only~~ of a frog, expose the heart by cutting away the body wall from directly over the ventricle, using care not to lose

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blood. Take a record of the movements of the ventricle, using a light straw lever of the fulcrum-power-weight order. Give **1.5cc** of **95 percent alcohol** in the lymph sac. Take a continuous record during the time of absorption. When much blood is lost and the circulation is poor it is better to apply the alcohol directly to the heart from an irrigating bottle, page 52. Irrigate the surface of the heart with **50 percent alcohol** in physiological saline.

Still more satisfactory results are obtained by perfusing the heart through a cannula in the ascending vena cava. Perfuse the alcohol from supply flasks provided with constant level tubes. The perfusion strength of **alcohol** to use is **2 to 5 percent** for 2 to 4 minutes at a time. Record the contractions of the ventricle by a thread from its tip to the vertical arm of a balanced lever, page 48. In this experiment, as in all frog's heart perfusions, it is better to use the weaker Ringer for the normal solution.

4. Alcohol on muscle work. Ligate one leg of a frog near the thigh to exclude its circulation. Inject **0.3cc** (5 minims) of **95 percent alcohol** in the dorsal lymph sac. In exactly 20 minutes pith the frog, and quickly prepare its alcoholized muscle covered with its skin, mount and determine the work it can do by stimulating the muscle directly with a single induction shock once every two seconds until it is completely exhausted. Record the contractions on a drum with a speed of 1 mm a second. Prepare the second or normal muscle, mount and stimulate in the same manner. Record the second experiment on the same smoked paper and parallel with the first. Repeat this experiment using a dose of **0.6cc** of **95 percent alcohol.**

5. Alcohol on voluntary work of human muscle. Demonstration. Measure the voluntary power of the flexors of the middle finger using Mosso's ergograph with a load of 3 kilos. Take normals at intervals of 15 or 20 minutes. Now take a dose of **20 to 40cc** of **20 percent alcohol.** Remeasure the muscular power after 60, 90, and 120 minutes respectively. Compute the work done in kilogrammeters.

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References: Lombard; Jour. Physiol., Vol. 13, p. 49; Hellsten; Skand. Arch. f. Physiol., Bd. 16, S. 139.

6. Alcohol on the circulatory and respiratory systems of the mammal. Anaesthetize a dog with morphine and chloroform, p. 46. Take the blood pressure from the carotid artery, p. 49, and the respiration from a side branch from a tracheal cannula. Expose the saphenous vein and insert a cannula for intravenous injections from a 50cc burette. Open the abdominal cavity, remove the outer sheath from the left kidney and enclose that organ in a renal onkometer. Record the kidney volume changes by means of a Brodie's bellows or Roy's piston recorder. The anaesthetic must be given with perfect regularity, 2 to 6 drops of chloroform every 30 seconds.

Take a record on the continuous kymograph and when all is in good working condition slowly inject **20 percent** warmed **alcohol** from the burette into the vein until some decided effect on the blood pressure is noted, i. e. after a dose of **20** to **40cc.** Extreme caution must be observed lest the heart be paralyzed and the animal die at once. The experiment should be repeated with different doses.

7. Alcohol on the reaction time of the reflex frog. Destroy the brain only of a frog and when it has recovered from the shock test the normal reaction time to electrical stimuli applied to the toe of the foot, page 51. Measure the time of the reaction with a watch or record with a writing point attached to the foot or leg of the suspended frog. Give a dose of **0.3cc** (5 minims) of **95 percent alcohol** in the dorsal lymph sac. Retest the reaction time at exactly 20 and 40 minutes after the injection. Compare the results with experiments 1 and 4 above.

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ETHER.

The effects of ether.

1. On the frog.
2. On the ventricular muscle.
3. On the frog's heart.
4. On the mammalian heart.
5. On the irritability of voluntary muscle.
6. On nerve tissue.
7. On the blood pressure and respiration of a mammal.
8. On the germination of seeds.
9. On the growth of yeast.

1. Ether on the frog. Inject into the dorsal lymph sac of a frog **0.2cc** (3 1-2 minims) of **ether.** Give **0.3cc** to a second frog. The first dose will produce anaesthesia in 8 to 10 minutes. Slight power of reflex response is retained. The voluntary motions will be regained in from 60 to 90 minutes if the animal is kept moist, and complete recovery in two hours. The larger dose will be recovered from in 20 to 24 hours or may even prove fatal.

2. Ether on the ventricular muscle. Mount a strip of terrapin's ventricle and establish rhythmic contractions in a bath of 0.7 percent saline. Record on a drum moving 1 to 2 mm per second. Immerse the strip in a bath of **1 percent ether** in saline for 2 to 3 minutes, then return to a physiological saline bath. The sharp decrease in both amplitude and rate of contractions is recovered quickly in the saline bath.

Repeat using **2, 4,** and **6 percent ether** solution. The weaker solutions occasionally produce an increase in rate, the initial excitation stage.

3. **Ether on the frog's heart.** A frog is pithed, its heart exposed, and a record taken by a simple lever on the ventricle. Record on a slow drum. Irrigate the surface of the heart with **0.7 percent saline solution** from an irrigating bottle, method page 48, and consider this record as the normal. Substitute a **saturated ether** solution in 0.7 percent saline for the irrigating liquid (about 8 percent by volume).

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Return to physiological saline irrigation after 3 to 5 minutes. Repeat after complete recovery. Solutions of ether applied to the surface of the heart rarely produce complete anaesthesia. The saline quickly restores contractions to twice and even thrice the original amplitude.

A second and more effective method is to insert a cannula in the inferior vena cava and perfuse the heart in place. Feed the heart first with 0.7 percent saline and follow with **1 percent ether** in saline. Repeat using **2 percent ether.**

4. Ether on the mammalian heart. Demonstration experiment. Use method as presented by Cushny, Jour. Exp. Medicine, Vol. II, page 233.

5. Ether on the irritability of voluntary muscle. Mount a gastrocnemius of the frog in the moist chamber, arrange to stimulate the muscle directly with a current of medium intensity but which produces a maximal contraction. Connect a vapor apparatus containing **1** or **2 percent ether** water with the gas tube of the moist chamber. Stimulate the muscle with single induction currents two times in quick succession, every 30 seconds through the entire experiment. Record on a drum having a rate of 2 mm per second. Take three or four normals then turn on the **ether vapor** for 5 minutes. Remove the vapor quickly with fresh air. The muscle's irritability will decrease to a point at which the stimulus is submaximal or even subminimal, but when the ether vapor is removed the contractions quickly reappear and attain their former amplitude.

One may readily demonstrate on this preparation that the muscle has a diminished power to do work when etherized.

6. Ether on nerve tissue. Prepare a muscle nerve of the frog isolating the entire sciatic with a piece of the cord and with the skin covering the muscle. Mount the preparation with the nerve in the moist chamber and on the electrodes but with the muscles hanging through the hole in the floor of the moist chamber and on the outside. Close the hole with moist filter paper. Proceed exactly as in

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experiment 5 above testing the irritability of the nerve through its effect on the muscle.

The influence of ether on nerve irritability may also be demonstrated directly by the action current method. For a description of the method read: Greene, Am. Jour. Physiol., Vol. I, p. 104.

7. Ether on the blood pressure and respiration of the mammal. Anaesthetize a mammal, introduce an arterial cannula in the carotid for blood pressure record. Insert a tracheal cannula and take a record of intra tracheal pressure from a T-tube attached to the cannula. Insert a cannula in the saphenous vein for injections. Give ether from a loose cloth thrown in a funnel about the end of the tracheal tube, or from an ether bottle. While taking a continuous record push the **ether,** then allow partial and guarded recovery. Repeat pushing the ether to the point where respirations cease. The blood pressure is an index of safety, for it has been shown that respiratory impulses are quickly re-established when the blood pressure remains high.

Also give **saturated ether** in saline as an intravenous injection in doses of **20cc** and more.

The rectal temperature should be recorded at intervals to demonstrate the fall of temperature under anaesthetics. Note also the state of dilation of the pupils.

8. Ether on the germination of seeds. Arrange two 8 ounce wide mouth bottles with stoppers fitted with two glass tubes letting one tube extend to near the bottom of the bottle. Suspend in each, by means of cotton, a dozen seeds—corn, wheat, clover, beans, etc.—and introduce just enough water to maintain a saturated vapor. Set both bottles in a window. Through one pass **ether vapor,** through the other **air,** twice a day for a week. The seeds in both will swell from the absorption of water, but only the bottle with pure air introduced will grow. Reverse the two. The sprouting grain will have its growth checked and the etherized seeds will begin to grow.

9. Ether on yeast. Take two fermentation tubes of active yeast culture, add five minims pure ether to one. Note the relative rate of gas liberation.

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CHLOROFORM.

The effect of chloroform.

1. On the frog.
2. On the heart strip.
3. On the frog's heart.
4. On muscle irritability.
5. On nerve.
6. On the blood pressure and the respiration of a mammal.
7. On the dog's heart.
8. On the kidney secretion.
9. On germinating seeds.

1. **Chloroform on the frog.** Inject **0.1cc** of **pure chloroform** or **0.3cc** of **20 percent chloroform** in olive oil, into the dorsal lymph sac of a frog. The anaesthesia is more profound and the recovery less rapid than in the case of ether.

2. **Chloroform on the heart strip.** Proceed as with ether in experiment **2,** page 8, using a **0.05 percent** solution of **chloroform** in 0.7 percent saline for one to three minutes. The contractions cease almost at once. Recovery in saline takes place very slowly. In comparison with ether the anaesthetic period is long. The amplitude of the first contractions to reappear is very slight and the rate slow and irregular. The original character of activity is not restored within 20 to 40 minutes.

An instructive picture is given by parallel records of experiments on the same strip of the effect of 4 percent ether and 0.1 percent chloroform for three minutes, both in saline.

3. **Chloroform on the frog's heart.** Proceed as in the similar experiment with ether, page 8, using **0.5 chloroform** in physiological saline to irrigate the outer surface of the heart. The amplitude is reduced to one half and the rate markedly slowed or entirely suppressed. Both are recovered when the chloroform is removed, but not so quickly as with ether.

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Perfuse the heart with **0.05 percent chloroform** in saline through the vena cava.

4. **Chloroform on muscle irritability.** See ether experiment 5, page 9. Use **0.1 percent chloroform water** in the vapor apparatus. Care must be used to remove the saturated chloroform vapor from the apparatus just before using, otherwise the muscle, or the nerve in the next experiment, will be over anaesthized and will not recover its irritability. There is no danger from ether from this cause.

5. **Chloroform on nerve.** Repeat ether experiment **6.** Use **0.1 percent chloroform water** in the vapor apparatus.

6. **Chloroform on the blood pressure and on the respiration of a mammal.** Proceed as with ether experiment **7,** page 10, using chloroform to anaesthetize the dog or cat (rabbits are too sensitive to chloroform for this experiment except in practiced hands). Remember that chloroform is said to be forty times as strong as ether in its effects. With extreme care chloroform anaesthesia may be pushed to the point where respirations cease and the animal recovered without artificial respiration. Often, however, in 5 to 10 second after respirations cease, the blood pressure will suddenly sink to a low level, and the heart will become weak and slow (see experiment **7** below), a state from which recovery can be secured only by quick and vigorous artificial respiration.

Give chloroform intravenously in doses of **10** to **20cc** of **0.5 percent** solution in saline, allowing plenty of time for recovery in each test. Compare with alcohol and ether.

7. **Effect of chloroform on the dog's heart.** Demonstration to show changes in both the auricle and the ventricle.

8. **Chloroform on the kidney secretion.** Anaesthetize a dog with morphine, 1cc of 1 percent, and chloroform, avoiding deep anaesthesia during the preliminary preparations. Insert a ureter cannula and connect it with a horizontal

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glass tube mounted on a graduated scale. Take a continuous record of the arterial pressure.

Now establish the normal rate of secretion of urine per 10 minutes for at least 40 minutes, then inject intravenously **10cc** of **0.5 percent chloroform** solution in saline. Double the dose if necessary until profound anaesthesia with low blood pressure and weak heart is obtained. Or produce deep anaesthesia by means of the respiratory inhalations. Recover and maintain light anaesthesia for an hour or more. The circulation is quickly re-established in good condition, but the secretion of urine which is suppressed during the deep anaesthesia stage is only slowly brought up to the normal with the re-establishment of good circulation.

9. Chloroform on germinating seeds. Repeat the experiment described for ether, page 10, passing air from saturated chloroform water into one bottle of seed and pure air into the other. After the seeds have sprouted, reverse the bottles. Both seeds and young growing plants are anaesthetized by chloroform. The seeds may not grow later as the drug kills plant protoplasm when given beyond a rather narrow limit of both time and concentration.

CHLORAL HYDRATE.

The effect of chloral hydrate.

1. **On the frog.**
2. **On the rabbit or cat.**
3. **On the heart.**

1. Chloral hydrate on the frog. Give a hypodermic injection of **0.5cc** of **2 percent chloral hydrate** in 0.7 percent saline. Keep in a moist battery jar until complete recovery. Give particular attention to effects on the nervous system.

2. Chloral hydrate on the cat or rabbit. Give a hypodermic injection of **2cc** of **2 percent chloral hydrate** in saline. Repeat in fifteen minutes if necessary to produce the chloral convulsions. Make close comparisons with the effects of strychnine, experiment 2, page 20.

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3. **Chloral on the heart.** Pith a frog and take tracings of the ventricle when irrigated with **1 percent chloral hydrate.**

THE OPIUM SERIES.

The effects of morphine.

1. **On the frog.**
2. **On the mammal.**
3. **On the ventricular muscle.**
4. **On the frog's heart.**
5. **On the reflex reaction time.**
6. **On the volume of air breathed.**

The effects of codeine.

7. **On the frog.**
8. **On the reflex reaction time.**

The effects of thebaine.

9. **On the frog.**
10. **The morphine group on the circulation and respiration in the mammal.**

1. **Morphine on the frog.** Give a dose of **1cc** of **2 percent morphine** in physiological saline in the dorsal lymph sac. Keep under observation for 2 to 3 hours to secure the later effects in the frog.

An instructive comparison is had by giving a dose of **0.5cc** (8 minims) of **0.1 percent strychnine nitrate** to a second frog at the same time.

2. **Morphine on the mammal.** Give a hypodermic of **1.5cc 2 percent morphine** to a 10 kilo dog. Keep the animal under observation for at least two hours and note the temperature, irritability, respiratory, occular, and other changes under the influence of the drug. Normals should, of course, be taken before giving the morphine.

3. **Morphine on the ventricular muscle.** Prepare a strip of the ventricle of a terrapin and get it into regular contractions by a bath of 0.7 percent sodium chloride. Change

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to a bath of **1 percent morphine** in physiological saline for five minutes when the strip should return to saline. Record on a drum moving 1 to 2 mm a second. This experiment shows that the cardiac contractions are weaker and slower under morphine. Repeat, varying the conditions according to the results of the previous experiment.

4. **Morphine on the frog's heart.** Pith a frog, expose the heart and take a record of its contraction. Test the irritability of the vagus trunk. If the circulation is still effective give a lymph sac injection of **0.5cc** of **10 percent morphine acetate** or apply drops of this solution directly to the heart from a dropping bottle. Retest the effectiveness of the vagus stimulation.

5. **Morphine on reflex reaction time.** Test the reaction time in a reflex frog in the usual way, first on the normal, then after a dose of **1cc** of **4 percent morphine.**

6. **Morphine on the volume of air breathed.** Anaesthetize a rabbit or cat with two grams urethane and ether, administering the latter at perfectly regular intervals and constant number of drops. Keep a continuous record of the respiration rate per minute either by counting or by recording on a cylinder. Measure the respiration volume as follows: Insert a tracheal cannula and connect it with an apparatus for measuring the volume of expired air. Ether can be given through the open tube of the apparatus except when actually measuring the air volume. Fill the graduated cylinder with water by means of the filter pump. Then measure the volume of four or five expirations and repeat for normal averages. Compute the expiration volume per minute. Now give **1cc** of **2 percent morphine** hypodermically. Remeasure the respiratory rate and expiration volume at intervals of 10 minutes.

7. **Codeine on the frog.** Give an injection into the dorsal lymph sac of **1cc** of **1 percent codeine** in physiological saline. Compare with morphine in experiment above.

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8. Codeine on the reflex reaction time. Repeat experiment 5 above with a dose of **1cc** of **1 percent** solution of **codeine** in the lymph sac.

9. Thebaine on the frog. Give an injection of **1cc** of **1 percent** solution of **thebaine** and compare with morphine and codeine in experiments 1 and 6 above.

10. The morphine group on the circulation and on respiration in the mammal. Anaesthetize a dog with chloroform (no morphine), take blood pressure from the carotid, insert a tracheal cannula and take respiration by the intratracheal method. Insert a cannula into the saphenous vein and connect with a burette for intravenous injections. (One may readily insert a cannula into the ureter and follow the secretion of urine under morphine. Consult the instructor.)

Give an intravenous injection of **2cc** of **0.5 percent morphine** in saline. The injected solution should be about the temperature of the body. Repeat after ten minutes using **4cc.** When equilibrium is re-established give **2cc** of **0.5 percent codeine.** Give **2cc** of **0.5 percent thebaine.** Give thebaine first if there is opportunity to make the test on a second animal.

The anaesthetic must be gradually diminished according to the amount of morphine etc. that has been injected. Use artificial respiration if necessary.

CAFFEINE.

The effects of caffeine.

1. **On the frog.**
2. **On the ventricular muscle.**
3. **On the frog's heart.**
4. **On the muscle irritability and muscle work.**
5. **On voluntary work of human muscle.**
6. **On the reaction time in man.**
7. **On the reflex reaction time in the frog.**
8. **On the mammalian heart.**

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9. On the circulation and the respiration in the mammal.

10. As a diuretic.

1. **Caffeine on the frog.** The dose is **1cc** of **0.5 percent caffeine** in the dorsal lymph sac. There is usually a great increase in irritability with muscle cramps in the later stages and final paralysis. Note the recovery stages when kept in a moist battery jar.

2. **Caffeine on the ventricular muscle.** Record the contractions of a strip of terrapin's ventricle beating in physiological saline on a slow speed drum. Change the solution to **0.1 percent caffeine** in saline for five minutes or less, then back to saline alone. Repeat, varying the time of the immersion in the drug. Increase the strength of the solution.

3. **Caffeine on the frog's heart.** Expose the heart of a pithed frog and adjust a balanced lever on the ventricle. Irrigate with physiological saline from a dropping bottle for a few moments, then change to **1 per cent caffeine** in saline for 5 minutes. Repeat once or twice then apply caffeine continuously until the maximum effect is obtained. Compare especially the auricle with the ventricle in the later stages.

When the heart is perfused then the solution should not be stronger than **0.1** to **0.2 percent** in **caffeine.**

4. **Caffeine on the muscle irritability and muscle work.** Lay a tight ligature around the thigh of a frog to close off the circulation in one leg. Give a dorsal lymph sac injection of **0.5cc** of **1 percent caffeine.** Allow 20 minutes for absorption then pith the frog and prepare the caffeinized gastrocnemius and, first determine its irritability by the minimal stimulus method, and second the amount of work it will do when stimulated directly once in two seconds until completely fatigued. Use a constant load of 50 grams. Prepare the normal gastrocnemius and test, first its irritability and second its amount of work. If the records are

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taken on the same paper one above the other, the comparison is very sharp.

5. Caffeine on voluntary work of human muscle. Measure the amount of work of the flexors of the middle finger by means of a Mosso's ergograph while lifting a 3 kilo weight. Repeat in 30 minutes. Consider these as normals. Take two cups strong coffee or **0.3 gram** of **caffeine** in warm water. Remeasure the work of the flexors as directed above 60 and 90 minutes after taking the caffeine. The amount of the muscular work is usually markedly increased by caffeine. See the next experiment.

6. Caffeine on the reaction time in man. Arrange a 100 double vibration tuning fork and a set of signal keys for measuring the reaction time to touch. Determine the normal reaction time, then the reaction time at 30, 60, 90 and 120 minutes after a dose of **0.3 grams caffeine** in warm water. This experiment may be performed together with the preceding.

7. Caffeine on the reflex time in a frog. Prepare a reflex frog by destroying the brain above the medulla. After the shock has passed away determine the reflex reaction time to electrical stimuli applied to the toe of the suspended frog. This time is easily recorded by a paper writing point attached to some part of the foot itself. Give a dose of **1cc** of **0.5 percent caffeine** in the dorsal lymph sac. After 30 minutes re-determine the reflex time. Draw conclusions from averages here, as the error of procedure is high.

8. Caffeine on the mammalian heart. Demonstration by Cushny's method.

9. Caffeine on the circulation and respiration of a mammal. Give a dog a hypodermic of **1cc** of **2 percent morphine** and anaesthetize with **chloroform.** Tracheotomize. Take arterial blood pressure from the carotid and respiration pressure from the trachea. Insert a cannula in the saphenous vein for intravenous injections. Take tracings on a continuous paper kymograph. After securing a nor-

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mal record give an intravenous injection of **5** to **10cc** of **0.2 percent caffeine** in physiological saline. Give from a warmed burette. This experiment should begin with small doses which should gradually be increased. See the next experiment on diuretic action.

10. Caffeine as a diuretic. Prepare a dog as in the preceding experiment. Take blood pressure and respiration rate. Insert a cannula for veinous injections.

Open the abdomen in the median line, seek out one ureter near its connection with the bladder, ligate and insert a urethal cannula, taking care to make an unobstructed connection. After the secretion flow has been established, a 2-3 mm rubber tube is connected with the cannula and the abdominal opening sewed up. Measure the flow of urine through a 2cc burette graduated to 1-50cc. Clamp the burette horizontally on a level with the kidney. Measure the column of urine each 10 minutes setting off the column by an injection of air from a hypodermic inserted through the rubber tube into the mouth of the burette. The normal secretion with medium light anaesthesia will vary, but ought to be 0.6 to 0.8cc in 10 minutes with a 10 kilo dog. Determine the normal secretion rate through at least 5 periods of 10 minutes each taking a continuous record of blood pressure and respiration rate. Give intravenous doses of **5cc** of **0.2 percent caffeine** in saline, at intervals of 20 minutes until a marked effect is produced on the amount of urine secreted. Then determine the rate of return to normal. Caffeine is one of the best diuretics, but an overdose will suppress the urine secretion often for many minutes. Refer to the diuretic action of salts.

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STRYCHNINE.

The effect of strychnine.

1. **On the frog.**
2. **On a mammal, demonstration.**
3. **On the ventricular strip.**
4. **On the frog's heart and cardiac nerves.**
5. **On reflex irritability and reaction time.**
6. **Local action on the spinal cord.**
7. **Spasms depend upon cutaneous stimulation.**
8. **Absorbed slowly from the stomach or bladder and readily from the intestine or peritoneum.**
9. **Stored in the spinal cord.**
10. **On the blood pressure and respiration rate of mammals.**

1. **Strychnine on the frog.** Give a frog a toxic dose of **strychnine nitrate, 0.3cc** (5 minims) of **0.1 percent.** Give in the dorsal lymph sac. Note the time of the appearance and the successive stages of increased irritability, convulsions, paralysis. Take fresh frogs and determine the limits of the therapeutic or non-toxic dose.

2. **Strychnine on the mammal. Demonstration.** Give a rabbit a hypodermic injection of **1cc** of **0.1 percent strychnine nitrate.** Consult Cushny's Pharmacology for symptoms. Meltzer gives the toxic dose for rabbits as 0.5 mgr. per kilo.

3. **Strychnine on the ventricular strip.** Suspend a strip of terrapin's ventricle in physiological saline and, when it is contracting, subject it to a bath of **0.1 percent strychnine nitrate** in saline for 5 minutes. Return to physiological saline.

4. **Strychnine on the heart and cardiac nerves.** Pith a frog, expose the heart, take a direct tracing of the movements of the ventricle. Test the vagus activity. Irrigate the heart from a dropping bottle with **0.1 percent strychnine nitrate** in physiological saline. While continuing the

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irrigation stimulate the vagus trunk at intervals of 5 to 10 minutes. Look for a progressive effect on the beat, the rate, and on the nervous mechanism.

5. Strychnine on reflex irritability and reaction time. Prepare a reflex frog, i. e. destroy the brain only. After the shock has passed away, one hour or more, determine the reflex reaction time to electrical stimulation of the toe by the method given on page 51. Now give **0.5cc** (8 minims) of **0.02 percent strychnine nitrate.** After each ten minutes take the reaction time to electrical stimultion until spasms appear, which ought to be under 40 to 60 minutes.

6. Local action of strychnine on the spinal cord. Cut the cord of a frog at the base of the medulla and destroy the brain. Paint the cut end of the cord with **0.1 percent strychnine nitrate** solution. Muscular spasms will be produced. Pith the frog, the spasms cease.

7. Strychnine spasms depend also upon cutaneous stimulation. Strychninize a frog and when the spasms are strong and continuous paint the skin with a **2 percent cocaine** solution. The cocaine paralyzes the cutaneous sensory apparatus whereupon the convulsions cease. Dip the frog in water to remove the excess of cocaine when its local effect will disappear in about 10 minutes and the convulsions will reappear.

8. Strychnine is absorbed very slowly from the stomach or bladder but very readily from the intestine and body cavity. Anaesthetize a fasting cat. Ligate both the cardiac and the pyloric orifices of the stomach. Inject **10cc** of **0.1 percent** (10 mgrs.) of **strychnine nitrate** into the stomach. If no spasms occur in 30 minutes then cut the pyloric ligature and run the stomach content into the intestine. Spasms may be looked for in 5 minutes or less. If enough strychnine is absorbed from the stomach to produce muscular spasms repeat on a second animal but inject the drug into a portion of the intestine.

Compare absorption from the urinary bladder and from the abdominal cavity in the same manner.

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9. **Strychnine is stored in the spinal cord** (Lovett: Jour. Physiol., Vol. IX, p. 99). Inject **10 mgrms. strychnine nitrate** in the dorsal lymph sac of a large bull frog. Allow 30 minutes for absorption. Then remove the skin and wash away all traces of strychnine that may remain unabsorbed. Take the cord, also an equal amount of other tissue, mascerate in 0.7 percent saline. Inject equal portions of the extracts in the dorsal lymph sacs of two frogs. Allow hours, if necessary, for the symptoms to develop. This method will detect traces of strychnine too small for chemical identification.

10. **Strychnine on the blood pressure and respiration rate of mammals.** Anaesthetize a 10 kilo dog. Take a continuous record of the blood pressure from the carotid and of respiration rate from the trachea. Give an injection of **1cc of 0.1 percent** solution of **strychnine nitrate** from a hypodermic into the saphenous vein. One should give close attention to the symptoms of this mild dose which will produce little more than the therapeutic effects. Repeat this injection until the cumulative dose produces convulsions of a mild character. Note that the convulsions may be suppressed by giving more chloroform.

COCAINE.

The action of cocaine.

1. **On the frog.**
2. **On local sensory surfaces.**
3. **On the heart muscle.**
4. **On the frog's heart.**
5. **On muscle work.**
6. **On the circulatory and respiratory systems of the mammal.**

1. **Cocaine on the frog.** Give a frog a dorsal lymph sac injection of **0.4cc** of **0.5 percent** solution of **cocaine hydrochlorate** in physiological saline. Examine the white corpuscles of the blood for motility as compared with the unpoisoned frog.

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2. Cocaine on local sensory surfaces. Paint one-half the surface of the tongue with a brush wet in **2 percent** solution of **cocaine hydrochlorate.** Use care not to swallow any of the solution. In 8 to 10 minutes compare the sensitiveness of the two halves of the tongue to electrical currents by the minimal stimulus method. Test for taste sensations of sweet, of salt.

Give one drop of **one percent cocaine** in the right eye; repeat in three minutes. There is a loss of sensitiveness and the eyeball may be touched without pain. Compare the pupils as to size, as to reaction to light. Determine the acuteness of vision of each eye separately at the reading distance. Cocaine is an analgesic but not a perfect midriatic.

3. Cocaine on the heart muscle. Prepare a strip of terrapin's ventricle and when it is beating in physiological saline in good rhythm submit it to a 3 to 5 minutes bath of **0.01 percent cocaine** in saline. Record the contractions on a slow drum.

4. Cocaine on the frog's heart and its nervous mechanism. Pith a frog, expose the heart. Take a continuous record of its contractions by the usual method. Irrigate its surface with physiological saline. Test the irritability of the vagus trunk using a medium strength stimulus. Now irrigate the heart for one minute with **0.2 percent cocaine** in saline followed by saline. When the cocaine contractions have somewhat recovered, retest the inhibitory power of the vagus. Repeat the test using 2 or 3 drops of **1 percent** solution and not washing it off afterward.

If the heart is perfused, then one should use a **0.01 percent** solution, preferably in Ringer's weaker solution.

5. Cocaine on muscle work. Ligate one leg of a frog at the thigh. Give a dose of **0.4cc** (7 minims) of **0.5 percent** cocaine in the dorsal lymph sac. Allow 20 minutes for absorption then rapidly prepare the cocainized gastrocnemius and take records of its contractions on a drum with a speed of 1 mm. per second. Stimulate with single break

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induction shocks once in two seconds until exhausted. Load with a 50 gram weight. Prepare the normal gastrocnemius of the ligated leg, mount and stimulate with the same rate and load. If the two records are parallel on the same paper it will demonstrate the comparative difference in work done. Calculate the amount of work per gram of muscle in each of the two preparations.

6. **Cocaine on the circulatory and respiratory systems of the mammal.** Give morphia and chloroform to a dog. Insert a tracheal cannula. Take blood pressure and respiratory records on the continuous paper kymograph. Insert a cannula and connect a burette with the saphenous vein. Inject **2cc** of **1 percent cocaine** very slowly and while watching the blood pressure as an indicator.

QUININE.

The effect of quinine.

1. **On the frog.**
2. **On the frog's heart.**

1. **Quinine on the frog.** Inject **1cc** of **0.1 percent** solution of **hydrochlorate of quinine** into the dorsal lymph sac. In addition to the usual observations, examine the blood of this frog as regards the motility of the white corpuscles. Compare with the blood of a normal frog.

2. **Quinine on the frog's heart.** Pith a frog, expose the heart and take a record of its contractions with physiological saline bathing from an irrigating bottle. Change the irrigating fluid to **0.1 percent quinine hydrochlorate** in saline for 5 minutes. Repeat after recovery using **1 percent quinine** in saline. Continue this irrigation until no further contractions are secured. Examine the condition of the ventricle.

Vary this experiment by pithing the frog, taking care to lose little blood and making a record from the ventricle. Now give a lymph sac injection of **1cc** of **1 percent quinine** and take a continuous record through 20 to 30 minutes.

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ATROPINE.

The action of atropine.

1. On the frog.

2. On the heart muscle.

3. On the frog's heart and the cardiac nervous apparatus.

4. On the secretory nerves of a mammal.

5. On the circulatory and respiratory systems in the mammal.

6. On the eye.

7. On the human in therapeutic dose.

8. Secreted by the kidney.

9. Hyoscyamine on the frog.

1. Atropine on the frog. Give a frog an injection of **1cc of 1 percent** of **atropine.** Keep in a moist battery jar until complete recovery. The toxic dose is **1cc of 5 percent.**

2. Atropine on the heart muscle. Mount a ventricular strip from the terrapin in 0.7 percent sodium chloride and when it is contracting with a uniform amplitude and regular rhythm change to **0.001 percent atropine** in physiological saline, for 5 minutes, Recover the characteristic rhythm in saline and repeat using **0.002 percent atropine** in saline.

3. Atropine on the frog's heart and cardiac nervous apparatus. Pith a frog, expose the heart and take a tracing. Determine an effective strength of stimulus for the vagus. Now irrigate the heart for one minute from a dropping bottle containing **0.1 percent atropine** in saline. Stimulate the vagus immediately and once every 5 minutes or less until all nervous effects are eliminated. The atropine effect is antagonized by physostigmine, page 33, experiment 3, and by muscarine.

4. Atropine on the secretory nerves of a mammal. Anaesthetize a 10 kilo dog with morphine and chloroform. Expose and tie a cannula in the parotid duct. Expose and stimulate the tympanic nerve in the hilus of the gland, noting the rate of secretion by the drops of saliva per min-

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ute from the cannula. Give a hypodermic injection of **1.5cc of 1 percent atropine.** Stimulate the tympanic nerve again. No secretion is obtained even though the nerve is stimulated down close to the hilus of the gland.

This demonstration may be made in part as follows: Produce a rapid flow of saliva in the dog by a hypodermic of **1cc of 0.2 percent pilocarpine.** Observe the flow by turning out the upper lip. Follow with a hypodermic dose of **1cc of 1 percent atropine.**

5. **Atropine on the circulatory and respiratory systems in the mammal.** Anaesthetize a 10 kilo dog as in experiment 4 preceding. Place an arterial cannula in the carotid and insert a tracheal cannula. Take a continuous record of the blood pressure and of the respiration. Stimulate the peripheral end of the sectioned vagus with a stimulus that produces complete inhibition of the heart. Stimulate also the central end of the vagus. Now give an intravenous injection of **1cc of 0.1 percent atropine.** Note the exact time of the injection on the record by a signal pen. When the equilibrium is again established, restimulate the ends of the sectioned vagus with the same strengths of stimulus used before atropine was given. Note that the pupil still actively dilates after this dose when the central end of the vagus trunk is stimulated. Atropine also destroys the nervous control over the smooth muscle of the alimentary tract thus decreasing its motility.

Physostigmine is an antagonist to atropine. Try **1cc of 0.1 percent** intravenous. Use artificial respiration if necessary. Give later a second injection of atropine.

6. **Atropine on the eye.** Drop **1 or 2 drops of 0.2 percent atropine** in the right eye of a dog or cat. The pupil will be widely dilated in a few minutes. Keep the animal under observation until the effect entirely disappears, often only after several days. Atropine destroys the power of accommodation and it is used for this clinical purpose in eye practice. Students should not use atropine on their own eyes, but a mild dose of **homatropine, 2** or **3 drops** of

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1 percent, the effect of which passes off in 24 to 36 hours, may be tested. In such experiment test the accommodation, light reflex, and size of the pupil.

7. **Atropine on the human in therapeutic dose.** Test on yourself the action of a dose of **1-120 to 1-60 grain atropine** by way of the mouth. Note the effects on the heart rate, pulse character, respiration, size of pupil, light reflex, sensations.

8. **Atropine is secreted by the kidney.** This may be demonstrated on the rabbit which is very tolerant of the drug. Give a rabbit urethane. Collect the urine from a bladder cannula. Give a large hypodermic injection, **2cc** of **2 percent atropine,** and test the urine on the eye of a cat or dog. The atropine may be extracted (Binz). Concentrate a large amount of urine, add ammonia, shake up with chloroform, evaporate, dissolve the residue and test on the eye of a cat or dog.

9. **Hyoscyamine on the frog.** Give a dose of **1cc** of **1 percent hyoscyamine** in the dorsal lymph sac of a frog. Compare with the effects of an equal dose of atropine in experiment 1 above.

NICOTINE.

Action of nicotine.

1. **On the frog.**
2. **On the ventricular muscle.**
3. **On the frog's heart and its nervous apparatus.**
4. **On nerve fibre and on nerve ganglia.**
5. **On the mammalian heart and the circulatory system and on the respiratory nervous mechanism.**
6. **On muscle irritability.**

1. **Nicotine on the frog.** Give an injection of a **0.5cc** of **0.2 percent nicotine** into the dorsal lymph sac of a frog.

2. **Nicotine on the ventricular muscle.** Prepare a terrapin's heart strip and when it is contracting rhythmically

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in 0.7 percent physiological saline, immerse the strip in a **0.02 percent solution** of **nicotine** in saline for 2 minutes, then return to the saline bath. Repeat.

3. Nicotine on the frog's heart and its nervous mechanism. Pith a frog, expose the heart and take tracings on a drum with a speed of 2mm per second. Stimulate the vago-sympathetic with an interrupted current that just causes complete inhibitions. Now irrigate the heart from a dropping bottle with **0.1 percent nicotine** in 0.7 percent saline, and stimulate the vagus at intervals of 2 minutes. If the nerve stimulation ceases to be effective, then apply the electrodes directly to the sinus.

To demonstrate the stronger effects on heart muscle prepare a second frog. Take a tracing of the heart. Apply a few drops of **1 percent solution of nicotine.**

4. Nicotine on the nerve and on the nerve fibre. (Read, Langley and Dickinson: Journal of Physiol., Vol. II, page 265.) Anaesthetize a rabbit (or cat or dog) dissect out the cervical sympathetic and the superior cervical ganglion. Stimulations of the nerve or of the ganglion lead to vaso constriction in the ear and dilation of the pupil. Paint the nerve below the ganglion with **1 percent nicotine.** Stimulation at a point still lower down shows that the nerve impulse still passes undisturbed. Now paint the ganglion itself. Stimulate the nerve below the ganglion, also the ganglion directly. Conclusions. See also the next experiment.

5. Nicotine on the mammalian heart and the circulatory and respiratory nervous mechanism. Anaesthetize a dog or cat (the animal used in experiment 4 above may be used for this experiment also). Take a blood pressure from the carotid and a respiration tracing from the trachea. Determine an effective stimulus for the heart and respiration. Now inject **5cc** of **0.1 percent** solution of **nicotine** into the saphenous or jugular vein. Repeat the dose if necessary, until distinct effects are produced on the heart rate and blood pressure. Then stimulate the vagus first with the strength of stimulus used before the injection, then with

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successively stronger stimuli. An instructive picture is obtained by dissecting down to and stimulating the cardiac branches from the annulus of Viusens, which may be done in the dog without opening the thorax,

6. **Nicotine on muscle irritability.** Lay a ligature around the thigh of one leg of a frog and then give **1cc** of **0.1 percent nicotine** in the dorsal lymph sac. After 20 minutes test the irritability of the normal and of the nicotinized gastrocnemius muscles by the minimal and maximal stimulus method.

CURARA.

The effect of curara.

1. On the frog.

2. On the motor nerve endings.

3. On the heart muscle and on the cardiac nervous mechanism.

4. Poisons the motor endings before the other portions of the reflex arc.

5. On the mammal.

1. **Curara on the frog.** Give a frog a hypodermic of **0.3cc** (5 minims) of **0.2 percent curara.** The motor apparatus is paralyzed but the circulation continues and the frog will recover in from one to three days, respiration being maintained through the moist skin if the animal is kept in a covered battery jar.

2. **Curara on the motor nerve endings, Bernard's experiment.** The specific action of curara was demonstrated by Claude Bernard to be on the motor nerve end plates. Ligate one leg of a frog to shut off the circulation, give a hypodermic of **0.3cc** of **0.2 percent curara.** When general paralysis is secured, perform the following tests, interpreting the results through the effect on the gastrocnemius muscles:

a. Stimulate the sciatic nerve on the unligated leg.

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b. Stimulate the gastrocnemius of this side.

c. Stimulate the sciatic nerve on the ligated side above the ligature.

d. Below the ligature.

e. The corresponding muscle. Conclusions.

3. Curara on the heart muscle and on the cardiac nervous apparatus. While minimal doses of curara suffice to poison the motor end plates it takes relatively large doses to paralyze the cardiac nervous apparatus. The paralysis apparently affects the ganglionic nerve endings first and then the cardiac motor endings and muscle. Pith a frog, expose the heart, prepare the vagus trunk for stimulation and adjust a heart lever for record. Allow physiological saline from an irrigating bottle to run over the heart. Take a normal record and then stimulate the vagus nerve. Now irrigate slowly with **0.2 percent curara** in saline and stimulate the nerve at intervals of 10 minutes for several tests.

4. Curara poisons the motor endings before the other portions of the reflex arc. Tie a ligature on a frog's leg, inject **0.3cc** of **0.2 per cent curara.** Just as voluntary activity ceases stimulate the skin of the poisoned leg. The unpoisoned gastrocnemius will contract. Rapidly expose and stimulate the poisoned sciatic. The poisoned gastrocnemius will not contract while the unpoisoned one will, owing to reflex stimulation through the cord.

5. Curara on the mammal. Morphinize and chloroform a dog. Take blood pressure. Introduce a tracheal cannula and take the respiratory record by the intratracheal method. Arrange the apparatus for artificial respiration when needed. Inject into a vein **5cc** of **1 percent curara.** All movements of voluntary muscles will quickly cease including respiratory movements. The heart rate and the blood pressure remain good and if artificial respiration is applied the circulation can be maintained for several hours.

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PILOCARPINE.

The action of pilocarpine.

1. **On the frog.**
2. **On the mammal.**
3. **On the ventricular muscle.**
4. **On the frog's heart.**
5. **On the circulatory and respiratory systems of the mammal.**
6. **On the secretory activity of the salivary glands.**

1. **Pilocarpine on the frog.** Give a frog a hypodermic injection of **0.6cc** of **10 percent** solution of **pilocarpine nitrate.** Keep the frog in a moist battery jar until normal again. Toxic dose, **1cc** of **10 percent** solution of **pilocarpine.**

2. **Pilocarpine on the mammal.** Give a dog a hypodermic injection of **0.3cc** (5 minims) **1 percent pilocarpine.** This dose produces a marked secretion by glandular structures. Examine the flow of saliva by turning back the upper lip, drying it and noting the accumulation of drops at the mouth of the salivary duct. Give additional drops of **1 percent pilocarpine** in the eye. Drops of **1 percent atropine** on the eye will overcome the action. An injection of **1cc** of **0.1 percent atropine** in a vein antagonizes **pilocarpine** and stops the secretion.

3. **Pilocarpine on the ventricular muscle.** Mount a strip of the terrapin's ventricle in physiological saline. When the contractions are regular transfer to a **0.1 percent pilocarpine** solution in saline. Allow it to act only 1 minute then renew the physiological saline bath. As a final test give the strip a continuous bath of **0.1 percent pilocarpine** and follow the successive effects.

4. **Pilocarpine on the frog's heart.** Pith a frog, expose the heart and take tracings of the ventricle. Test the activity of the vagus with a strong interrupted current. Now irrigate the surface of the heart with drops of **1 percent pilocarpine** for 2 minutes. Stimulate the vagus trunk 1 minute after pilocarpine and at successive intervals of 5 min-

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utes. If the drug is strongly active the stimulation will result in acceleration but no inhibition as in the normal. Applying the electrodes directly to the sinus gives an inhibition showing that the pilocarpine has acted on the vagus ganglionic connections. Drops of **1 percent atropine sulphate** will restore the heart beat after pilocarpine, these two drugs being antagonists.

5. Pilocarpine on the circulatory and respiratory systems of the mammal. Anaesthetize a dog with morphine and chloroform. Introduce a tracheal cannula and be prepared to use artificial respiration if necessary. Take blood pressure and respiration records on a continuous paper kymograph. Give an intravenous injection of **1cc of 1 percent pilocarpine nitrate.** Test the activity of the vagus before and after the pilocarpine. Atropine antagonizes the pilocarpine effect. Examine the pupils from time to time. Also note the increased rate of salivary secretion.

PHYSOSTIGMINE.

The action of physostigmine.

1. **On the frog.**
2. **On cardiac muscle.**
3. **On the frog's heart and its nervous apparatus.**
4. **On the respiratory medullary center and on the circulatory system of the mammal.**
5. **On the eye.**

1. Physostigmine on the frog. Give a frog a dorsal lymph sac injection of **1cc** (17 minims) **1 percent physostigmine.** The effects produced are diminished irritability, loss of muscular tone, paralysis of the respiratory center, loss of reflexes, and death, or at best a very slow and prolonged recovery.

2. Physostigmine on cardiac muscle. After a ventricular strip from the terrapin has begun beating regularly in physiological saline, transfer it to **0.1 per cent physostigmine** in saline for 2 to 3 minutes. Physiological saline re-

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covers the normal contractions after several minutes. Compare the results with those from pilocarpine and muscarine.

3. **Physostigmine on the frog's heart and its nervous apparatus.** Pith a frog and take a record of the heart beat. Determine the minimal effective stimulus of the vagus trunk for inhibition of the heart rate. Now irritate the surface of the heart with drops of **1 percent physostigmine** from an irrigating bottle for 2 minutes. Redetermine the minimal stimulus for the vagus trunk beginning with a very weak induction current. If the contractions are at a slow rate or have ceased, irrigate the surface of the heart with **1 percent atropine.** Atropine antagonizes physostigmine. Compare with pilocarpine.

4. **Physostigmine on the respiratory medullary center and on the circulatory system of the mammal.** Anaesthetize a dog, insert a tracheal cannula and be prepared for artificial respiration. Take a continuous record of the blood pressure and of the respiratory movements. Insert a canula in the saphenous vein and connect it with a burette containing the drug. Give an intravenous injection of **0.1 percent physostigmine** slowly until the first effects are noticed on blood pressure. Note the amount and mark the time of the dose on the record by a signal pen. Usually there is a progressive paralysis of the respiratory center accompanied by a great showing of the heart and a fall of blood pressure by one-half or more. The heart continues to beat long after respiration ceases. Use artificial respiration until the blood pressure improves and the anaesthesia becomes light. This will not restore automatic respiratory movements as it does after heavy anaesthesia. If at this time a venous injection of **0.5cc** of **1 percent atropine** be given from a hypodermic syringe the respiratory movements will be quickly established and the slow heart and low pressure will give way to the rapid heart and strong pressure following primary injection of atropine, experiment 5, page 26. The vagus inhibitory apparatus is effective under physostigmine but not after atropine. Repeat the experiment. It

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takes a larger dose of physostigmine to overcome the atropine and produce the characteristic effects. Examine the pupil before and after the physostigmine.

5. Physostigmine on the eye. Give **2 drops** of **1 percent physostigmine** in one eye of a dog or a rabbit, at intervals of 5 minutes. It is better to use one of the experimenter's own eyes. Strong contraction of the pupil follows. A decrease in intraocular pressure has also been proven, and to produce this effect is the chief therapeutic use of the drug. A striking comparison is obtained by dropping **1 percent atropine** in the unused eye of the dog after the physostigmine effect has come on. Physostigmine will overcome the atropine dilation of the pupil. The experimenter may show the antagonism between homatropine and physostigmine on his own eyes, but it is recommended that one eye always be reserved.

ACONITE.

The action of aconite.

1. **On the frog.**
2. **On the circulatory system of a mammal.**
3. **On the frog's heart.**

1. Aconite on the frog. The dose is **0.5cc** of **0.1 percent aconitine.** Compare with digitalis.

2. Aconite on the circulatory system of the mammal. Take a continuous tracing of the blood pressure of a dog. Give **1cc** of **0.1 percent aconitine** crystals. Note particularly the progressive effects on the nervous and muscular elements of the circulatory apparatus.

3. Aconite on the frog's heart. Destroy the cerebrum and optic lobes only of a frog, expose the ventricle and take a tracing. Give an injection of **0.5cc** of **0.1 percent aconitine** in the lymph sac. One may expect a progressive stimulation of the accelerator and vagus nervous apparatus followed by paralysis of nerves and muscle.

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VERATRINE.

The action of veratrine.

1. On the frog.

2. On the mammal.

3. On the frog's heart.

4. On the form of the simple muscle contraction.

5. On the circulatory and respiratory systems of the mammal.

1. **Veratrine on the frog.** The dose for a frog is about **0.5cc** of a **1 percent** solution of the **fluid extract veratrum viride** or **0.5cc** of **0.05 percent veratrine.** Compare with the effects of aconite and of barium.

2. **Veratrine on the mammal.** Give a cat or rabbit **1cc** of **0.1 percent veratrine** hypodermically, or **1cc** of **1 percent** for a dog.

3. **Veratrine on the frog's heart.** Pith a frog, expose the heart and take a tracing under irrigation with **0.1 percent veratrine.**

4. **Veratrine on the simple muscle contraction of the frog.** Ligate one leg of a frog and give a hypodermic of **0.5cc** of **0.1 percent veratrine.** After 15 minutes prepare the veratrinized muscle and take simple muscle contractions to show the form of the wave, using a tuning fork to record the drum speed. Compare this curve with that of the undrugged muscle. The frog of experiment 1 may be used to show the veratrine effect on muscle.

5. **Veratrine on the circulation and respiration of a mammal.** Take a record of the blood pressure from the carotid of an anaesthetized dog. Tracheotomize and take respiratory tracings. Give **1cc** of **1 percent veratrine** in the abdominal cavity. When marked cardiac slowing appears cut the vagi.

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DIGITALIS.

The action of digitalis.

1. **On the frog.**
2. **On the ventricular muscle.**
3. **On the frog's heart.**
4. **On the atropinized frog's heart.**
5. **On the mammalian heart.**
6. **On the circulatory and respiratory systems of the mammal.**
7. **Digitalis as a diuretic.**

1. **Digitalis on the frog.** Give a dose of **0.5cc** of **0.2 percent** soluble **digitalis.** The digitalis effects develop slowly. Note the heart rate and particularly the circulation in the web. Keep in a moist battery jar.

2. **Digitalis on the ventricular muscle.** Treat a strip of terrapin's ventricle contracting in saline to a bath of **0.001 percent digitalis** in saline. Follow with pure saline. Repeat with a **0.002 percent digitalis.**

Digitalis solutions may be used made up in the weaker Ringer, but as the rate and amplitude is very different from that in sodium chloride solutions the picture will be quite different though the same in kind. Delirium cordis of the strip is produced by the stronger solution acting for several minutes.

3. **Digitalis on the frog's heart.** Pith a frog and take a record of the contractions of the ventricle when irrigated with physiological saline. Irrigate slowly with drops of **0.2 percent digitalis** for 2 minutes then wash off with saline.

A more effective method is to perfuse the heart from a cannula in the vena cava (Walden: Am. Jour. Physiol., Vol. III, p. 123). Use a much weaker solution for perfusion, i. e., **0.0005 percent digitalis** in saline.

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4. Digitalis on the atropinized frog's heart. Atropinize the frog's heart to eliminate the cardiac nervous control, then repeat experiment 3 above.

5. Digitalis on the mammal heart. Demonstration. Use the Cushny method.

6. Digitalis on the circulatory and respiratory systems of the mammal. Anaesthetize a dog and take continuous kymographic records of the blood pressure and of the respiration. Slowly inject into the saphenous vein **2cc** doses of **0.5 percent digitalis** at 5 minute intervals until the three stages of digitalis effect on the heart and blood pressure are obtained. The anaesthetic must be perfectly constant. One may give the maximal dose of **4cc** of **1 percent** digitalis at once. In this instance the three stages are passed through rapidly and the animal will die in 10 to 20 minutes. Read Cushny's Pharmacology, pp. 430-435.

7. Digitalis as a diuretic. Morphinize and chloroform a dog. Take the blood pressure. Isolate the ureters near the bladder and insert cannulas using care not to occlude the ureters by twisting or otherwise. Connect the ureters by means of a T-tube with a horizontal 2cc burette graduated to 1-50cc. Close the abdomen with sutures. Insert a venous cannula and connect with a transfusion burette. Establish the normal secretion per 10 minutes cutting off the column of secreted urine by injecting a bubble of air into the mouth of the burette by inserting a hypodermic needle through the rubber connecting tube. Now inject **5cc** of **0.1 percent digitalis** or **strophanthin** into a vein and take the secretion in successive 10 minute periods until the flow is constant. Repeat the dose once or twice at long intervals. Mark the secretion intervals on the blood pressure record.

Compare these results with those observed on other diuretic drugs—caffeine, urea, inorganic salts, etc.

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ERGOT.

The action of ergot.

1. **On the frog.**
2. **On the heart muscle.**
3. **On the arterioles of the frog.**
4. **On the blood pressure and heart rate of a mammal.**

1. **Ergot on the frog.** Give **0.5cc** of the fluid extract.

2. **Ergot on the heart muscle.** Change a contracting heart strip from saline to a **10 percent** solution of the fluid extract of ergot in saline solution. Allow it to act for 5 minutes. Take a continuous record.

3. **Ergot on the arterioles of the frog's web.** Wrap a frog in a wet cloth and fasten to a frog board for examining the web. Give a lymph sac injection of **0.5cc fluid extract** of **ergot.** Select a good field of small arterioles and measure their diameter at once. Remeasure at intervals of 5 minutes as the ergot is absorbed.

4. **Ergot on the blood pressure of a mammal.** Give an intravenous dose of **0.5cc fluid extract** of **ergot** to a mammal while taking a record of the blood pressure.

SUPRARENAL GLAND.

The commercial preparation of the active principle of suprarenal gland, adrenalin hydrochloride, presents the same physiological action as the gland extract and has the special advantage of preparation in definite and known strengths. It has come into general use for therapeutic purposes and is, therefore, used in these experiments.

Action of adrenalin hydrochloride.

1. **On the frog.**
2. **On the ventricular strip.**
3. **On the frog's heart.**
4. **On the simple muscle contraction.**
5. **On muscle work.**
6. **On local mucous surfaces.**
7. **On the size of the blood vessels in the frog's web.**
8. **On general blood pressure and peripheral vasoconstriction.**

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1. **Adrenalin on the frog.** Give 0.5cc·0.1 percent in the dorsal lymph sac.

2. **Adrenalin on the ventricular muscle.** Transfer a terrapin's ventricular strip contracting in physiological saline to **0.01 percent adrenalin** in saline. Change after 2 to 5 minutes. The drum speed should be 1 cm per minute. Suprarenal extract has also been shown to increase the amplitude and the rate of the ganglion free ventricular muscle of the dog. Cleghorn: Amer. Jour. Physiol., Vol. III, p. 2773.

3. **Adrenalin on the frog's heart.** Use the perfusion method, page 48, with the heart in place and the inflow cannula in the ascending vena cava. Follow physiological saline perfusion with **0.001 percent adrenalin hydrochloride** in saline. The drum speed should be 2 mm per second. Direct application to the surface of the heart requires a strength of at least 0.05 percent adrenalin hydrochloride.

4. **Adrenalin on the simple muscle contraction.** Ligate one leg of a frog and give **0.5cc** of **0.05 percent adrenalin.** Allow 10 minutes for absorption. Compare the simple muscle contractions of the two gastrocnemii as regards **a,** amplitude; **b,** the time of the simple contraction. The muscle power of patients with Addison's disease has been shown to be greatly improved by giving the extract of suprarenal gland.

5. **Adrenalin on muscle work in the frog.** Prepare a frog as in experiment 4 above and test the work performed by the two muscles. For method see page 51.

6. **Adrenalin on mucous surfaces.** Paint one-half the tongue with **0.1 percent adrenalin hydrochloride.** At intervals of 5 minutes drop **0.01 percent** in saline (sterilize by boiling) in one eye. Compare the two halves of the tongue and the two eyes as to vascular condition. Examine the size of the pupils. Test for possible differences as to the sensitiveness of the conjunctiva. Try the haemostatic effect in the next operation you perform on a mammal.

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7. **Adrenalin on the size of the blood vessels of the frog's web.** Use a dose of **0.5cc** of **0.1 percent** as a hypodermic. See Ergot, Exp. 3, page 38, Nitroglycerine Exp. 3, page 41. Or apply drops of **0.1 percent** directly to the web.

8. **Adrenalin on general blood pressure and on vaso-constriction in a mammal.** Prepare a dog for blood pressure. Adjust an onkometer to the left kidney and record the change in volume with a Brodie's bellows or Roy's piston recorder. Give **2** to **4cc** of **0.01 percent adrenalin hydrochloride** slowly in a vein. Give **2cc** of **0.1 percent atropine** to eliminate the vagus action on the heart and repeat the adrenalin injection. Compare with digitalis, page 36, ergot, page 38, veratrine, page 35. Drugs of antagonistic action are, nitrites, page 41, potash salts.

NITROGLYCERINE AND THE NITRITES.

Action of nitroglycerine and the nitrites.

1. **On the frog.**
2. **On the heart muscle.**
3. **On the arterioles of the frog.**
4. **On the circulatory system.**

Nitroglycerine and the nitrites affect primarily the peripheral circulation causing vaso-dilation with fall of blood pressure. The specific action is on the muscular tissue.

1. **Nitroglycerine on the frog.** Give a frog a dose of **0.5cc** of **0.1 percent nitroglycerine** in the dorsal lymph sac.

2. **Sodium nitrite on the heart muscle.** Test the action of **0.02 percent sodium nitrite** on the contracting ventricular strip.

3. **Nitroglycerine on the arterioles of a frog.** Bind a frog for the examination of the web circulation. Then give **1cc** of **0.1 percent nitroglycerine** in the lymph sac. Imme-

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diately measure the smaller arterioles in a favorable field and re-examine every two minutes as absorption progresses. Try direct application of drops of 0.1 percent to the web.

4. **Sodium nitrite on the circulation volume.** Pith a frog or small terrapin. Insert a cannula in the aorta, or one of its branches, snip the veins with the scissors to allow free perfusion, set the frog board at an angle to facilitate drainage of liquid. Perfuse the blood vessels with the weaker Ringer's solution for a normal. Follow with **0.01 percent sodium nitrite** in the weaker Ringer keeping a uniform pressure of the perfusion liquids of from 6 to 10 centimetres. Measure the perfusion rate in drops per minute, or collect in a 25cc graduate.

Test the outflow when irrigated with 0.0005 percent soluble digitalis and follow with 0.001 percent sodium nitrite, both in the weaker Ringer.

5. **Amyl nitrite on the pulse.** Take normal pulse records with one of the standard sphygmographs. Break an amyl nitrite pearl on a handkerchief and breathe deeply the fumes. Pulse tracings taken 5 and 10 minutes later will show the usual signs of dilated blood vessels with accompanying low pressure. Slight headaches sometimes follow the use of amyl nitrite.

6. **Nitrites on mammalian blood pressure.** Anaesthetize and take the blood pressure of a dog. Give intravenous doses of nitrites in the following order repeating with larger doses if necessary and always allowing full time for recovery: **1cc** of **0.1 percent nitroglycerine, 3cc** of **0.1 percent; 2cc** of **0.1 percent amyl nitrite; 6cc** of **0.1 percent sodium nitrite.** The blood pressure remains low for a long time after sodium nitrite. A dose of **2** to **5cc** of **0.2 percent digitalis** or **2cc** of **0.01 percent adrenalin hydrochloride** will antagonize this effect.

Give 2cc of 0.1 percent atropine and repeat the above doses of nitroglycerine and sodium nitrite.

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CARBOLIC ACID.

The action of carbolic acid.

1. On the frog.

2. On the growth of yeast and bacteria.

3. On the circulatory and respiratory systems of a mammal.

1. Carbolic acid on the frog. Give a dose of **1cc** of **1 percent.**

2. Carbolic acid on the growth of yeast and of bacteria. Prepare six fermentation tubes of active yeast culture and as many test tubes of inoculated bouillon. Keep one tube of each for a normal and to the others add enough **10 percent carbolic acid** to make a series of 0.1, 0.5, 1, 2, and 4 percent solutions. Keep at laboratory temperature and observe through a period of several days.

3. Carbolic acid on the circulatory and respiratory systems of the mammal. While taking records of blood pressure and respiration by the usual method give an intravenous injection of **10cc** of **0.5 percent carbolic acid.** When the collapse stage is far advanced transfuse **1 percent sodium sulphate** slowly. Judge the amount by the action in overcoming the carbolic acid effects.

POTASSIUM SALTS.

Action of potassium salts.

1. On the heart muscle.

2. On the reaction time in the reflex frog.

3. On muscle irritability and muscle work in the frog.

1. Potassium chloride on the heart muscle. A ventricular strip contracting in physiological saline solution is transferred to **0.03 percent potassium chloride** in saline for 2 to 5 minutes. Contradictions return in saline even after stronger does of potash.

2. Potassium bromide on the reaction time in the reflex frog. Compare the reaction time of a reflex frog be-

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fore and 20 to 40 minutes after **0.3cc** of **5 percent potassium bromide** in the dorsal lymph sac.

3. **Potassium chloride on muscle irritability and muscle work in the frog.** Compare the two gastrocnemii as to irritability and as to amount of muscle work done. Dose **0.3cc** of **5 percent** hypodermic with one leg ligatured.

CALCIUM SALTS.

Action of calcium salts.

1. **On heart muscle.**
2. **On the frog's heart.**
3. **On the blood pressure and the respiration in the mammal.**

1. **Calcium chloride on heart muscle.** Transfer a ventricular strip from physiological saline to **0.03 percent calcium chloride** in saline for 3 to 5 minutes. Record on a drum speed of 2 cm per minute. Repeat using **0.06 percent.** The rate is increased and the amplitude often doubled. The stronger solution produces great increase in tone which often passes into delirium cordis. Potash salts antagonize. Read Ringer: Jour. Physiology, 1883.

2. **Calcium chloride on the frog's heart.** Perfuse the frog's heart through the vena cava with 0.7 percent sodium chloride and follow with **0.03 percent calcium chloride** in 0.7 percent sodium chloride. Recover the sodium chloride type of contractions then perfuse with 0.01 percent barium.

3. **Calcium chloride on the blood pressure and the respiration in the mammal.** Give an intravenous dose of **10cc** of **1 percent** for a dog. Cut the vagi and repeat the dose. Alternate with potassium **2** to **4cc** of **1 percent.**

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BARIUM SALTS.

Action of barium salts.

1. **On the frog.**
2. **On the heart muscle.**
3. **On the circulation and on the respiration movements in mammals.**

1. **Barium on the frog.** Dose, **1cc** of **1 percent of barium chloride.**

2. **Barium chloride on the heart muscle.** Transfer a contracting ventricular strip from 0.7 percent sodium chloride to **0.01 percent barium chloride** in saline. Short immersions increase the rate but long baths show that this salt does not sustain contractions as do calcium salts. A 0.1 percent solution in saline delays contractions with prevention of relaxation. Contractions still take place in 1 percent barium chloride. Compare with digitalis.

3. **Barium chloride on the circulation and on respiratory movements in mammals.** The effect on the heart and blood pressure and on respiration in a mammal is demonstrated by an intravenous dose of **5cc** of **0.2 percent** given slowly. This dose should be repeated several times both before and after section of the vagi. Barium salts act as strong poisons to the nerve centers, especially those in the medulla.

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OPERATIONS, APPARATUS AND SPECIAL METHODS.

PHYSIOLOGICAL SOLUTIONS.

The lymph and blood plasma in which the tissues develop are the true physiological solutions.

Artificial solutions imitate lymph in its isotonicity, its physical character, and in its composition, its chemical character. Sodium chloride in 0.6 percent solution, used first by Nasse in 1869 on frog's muscle, and by Bowditch in 1871 on the frog's heart, was supposed to prevent injurious changes in the tissue by virtue of its isotonicity. Ringer in 1883, and Locke in 1885, introduced the solutions which bear their names. They showed that the chemical factors play a fundamental part in the effects of these solutions on the tissues. At the present time we recognize that exact isotonicity is not nearly so fundamental as at first supposed and that these solutions are chemically active in relation to the living protoplasm.

1. **Physiological salt solution** or **normal saline.** Sodium chloride in distilled water 0.7 percent. More exact isotonicity is secured by 0.6 percent for frogs, 0.7 percent for terrapin and 0.9 percent for mammals.

2. **Ringer's solution.** The Ringer's solution that imitates the blood serum in its effects on heart tissue is made up in this laboratory in the following proportions:

    Sodium chloride, 0.7 percent.
    Potassium chloride, 0.03 percent.
    Calcium chloride (cryst.), 0.026 percent.

For heart work where a more rapid rate is desired the amount of potassium must be reduced to that in Ringer's original formula:

    Sodium chloride, 0.7 percent.
    Potassium chloride, 0.01 percent.
    Calcium chloride (cryst.), 0.026 percent.

3. **Locke's solution.** Locke's solution is a mixture of

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the salts in Ringer's solution with dextrose added to make 0.1 percent:

Sodium chloride, 0.7 percent.
Potassium chloride, 0.03 (or 0.01) percent.
Calcium chloride (cryst.), 0.026 percent.
Dextrose, 0.1 percent.

ANAESTHESIA.

The mammals usually available for laboratory experimental purposes are dogs, cats, rabbits and guinea pigs, each of which can best be anaesthetized by a special treatment of its own.

1. **Dogs.** Give a 10 kilo dog **1cc** (17 minims) of **2 percent morphine** under the skin of the shoulder, holding its head firmly between the operator's legs while the hypodermic injection is being given. Allow 10 to 15 minutes for the morphine to take effect. The morphine should be followed by **chloroform.** Give it by means of a small nose hood made by sewing a cheese-cloth, that has been folded in the form of a blunt cone, to a wire ring. When the voluntary movements have about ceased, tie the dog to a holder and take it to the experimental table. The tests of good anaesthesia are loss of voluntary movements, no cutaneous reflexes, slight corneal reflexes or none in deep anesthesia, even and fairly deep respiration, medium blood pressure and pulse. This condition of **anaesthesia is maintained by giving chloroform from a dropping bottle at absolutely regular intervals of 30 seconds by the watch.** The number of drops necessary for each animal will quickly be found by trial. In the experience of this laboratory it is from 3 to 6 drops per 30 seconds. The success of most pharmacological experiments on dogs depends upon maintaining an absolutely even anaesthesia.

Cats. A mixture of equal parts of **chloroform** and **ether** is the most practical anaesthetic for cats. These animals are anaesthetized most conveniently by putting them in a box of about two cubic feet dimension and provided with a close cover. A very convenient box is the tin cracker dis-

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play box with glass window obtained of the grocer. Drop in the box with the cat a small strip of cheese-cloth saturated with chloroform-ether mixture, 10 cubic centimetres in broken doses will anaesthetize a cat in 10 minutes. As soon as the animal falls down under the influence of the anaesthetic it should be taken from the box, fixed in the holder, and the anaesthetic given from a cloth in the manner and with care precribed above for the dog. Cats do not survive pure chloroform in the hands of the ordinary student anaesthetist.

Rabbits. Give rabbits **2 grams** of **urethane** by the mouth. Follow with light and careful use of **ether.** Or pure ether may be given without the urethane. Give the ether in the manner and with the regularity recommended above for giving chloroform to dogs. Do not use chloroform or even chloroform mixtures with rabbits.

Guinea pigs. These little animals when they must be used for pharmacological purposes are anaesthetized best with pure ether or ether followed with a little morphine.

THE PREPARATION OF THE VENTRICULAR MUSCLE.

Destroy the brain of a terrapin, remove the plastron and open the pericardium. Grasp the left angle of the base of the exposed ventricle with a forceps and cut with a scissors from this point around the apex to the opposite side thus removing a piece about one inch long and the size of the half of a small lead pencil. Split this strip into two or three smaller ones for class use.

To mount the heart strip tie silk threads to each end, one with a loop one half inch and the other with a loop about four inches long. Place the short loop on the hook of the glass rod holder provided for the purpose, and the long one over the recording lever. Use a straw lever of the power-fulcrum-weight order mounted in a muscle lever holder. A total tension of one gram is best for developing the contractions of the ventricular strip. The holder mentioned above is made of a glass rod 4-5mm diameter and 6 inches long. Bend it at a right angle in the middle and then

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draw out and turn a hook on one end, the hook being turned back on the rod. The apparatus set up complete consists of a single iron stand with three clamps, the top one to support the lever holder, the middle the glass rod, and the bottom one a platform on which rests the footed test tube to contain the solution surrounding the strip. The ventricular strip mounted in this apparatus with a tension of one gram and bathed in a solution of 0.7 percent sodium chloride will begin rhythmic contractions in from 20 to 40 minutes. These contractions will continue about two hours growing constantly smaller for the entire time. The strip may be revived by a bath of Ringer's solution or by serum, and may then again be used in the sodium chloride bath.

TO TEST THE ACTION OF DRUGS ON THE FROG'S OR TERRAPIN'S HEART.

Two methods are used in this laboratory for the study of the action of drugs on the frog's heart, both permitting of permanent records. The most convenient method is to pith the frog, open the thorax and expose the heart, adjust the foot of a delicately poised heart lever on the ventricle, and, while the record is being taken, irrigate the surface of the heart with the drug. Dissolve the drug in physiological saline and always take a previous normal record under saline irrigation. This method requires the use of relatively strong solutions. The most convenient irrigating bottles are four or eight ounce aspirator bottles with tubed foot for rubber hose. These are each provided with a small mouthed cannula attached by a short rubber connection, and the flow is regulated by a screw compress. Fit these flasks with Marriotte stoppers and support them on a stand by a universal burette clamp about the neck.

The second method, that of perfusion, is carried out best as described by Walden in the American Journal of Physiology, vol. 3, page 123. Insert a cannula into the inferior vena cava for an inflow and one in the aorta for an outflow. The former is connected with two supply bottles, one

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for physiological saline, the other for the drug in solution. The Marriotte stoppers should be set at exactly the same pressure levels. Connect the two flasks with the inflow cannula by a T-tube brought as close as possible to the heart in order that the solutions may be changed quickly with only a short connecting tube to be washed out. Very weak solutions of drugs are required by this method of perfusion. The frog's heart is quickly exhausted in pure saline solutions, so for certain experiments it is better to use the weaker Ringer's solution for dissolving the drug.

Record the contractions of the ventricle by a thread from its apex to the vertical arm on a balanced horizontal lever. A flexible paper writing point will add to the accuracy and beauty of the records.

TO TEST THE ACTION OF DRUGS ON THE BLOOD PRESSURE, RESPIRATION, ETC., OF A MAMMAL.

1. **The anaesthetic.** For anaesthesia methods see page 46.

2. **The operations.** Blood pressure is taken from one of two arteries, the right common carotid, or the femoral artery. The femoral is practical only for the dog. To expose the **common carotid** make a three inch cut over the trachea from the hyoid cartilage toward the manubrium. Separate the muscles down to the trachea, and then along the side of the trachea till the common carotid artery and vagus come into view. Use the scalpel handle and tear rather than cut the facias and muscles involved. Avoid the veins or the laryngeal arteries. No blood need be lost after the skin is cut. Separate the facia binding the artery and vagus using care not to injure the latter. Place a bulldog forceps on the artery well toward the thorax. Ligate the cephalic end. Lay and tie loosely a ligature about the intervening stretch of artery for the cannula. Grasp the artery at the cephalic ligature and use the tip of the scissors to make a V-shaped cut two-thirds through the artery wall

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and directed toward the heart. Insert the cannula and ligate it firmly.

The **femoral** artery is exposed by a 5 cm cut over the artery where the pulse can be felt near Poupart's ligament. The artery is prepared and the cannula inserted as described for the carotid.

The **saphenous** vein or the **jugular** are used for injecting drugs. Insert a small washout cannula toward the heart choosing the vein exposed by the previous operation. Keep the vein closed with a bulldog forceps in order to prevent small clots in the mouth of the cannula except when injections are to be made.

Tracheotomy should be performed in all student work on the mammals used in blood pressure experiments in pharmacology. Free the trachea immediately below the thyroid cartilage and insert a metal cannula made especially for the dog, or insert one limb of a glass T-tube of as large size as the trachea will take. Tie firmly with small twine.

The apparatus consists of a continuous paper kymograph (Ludwig's weight-driven pattern changed to run the paper in the right handed direction is the most satisfactory instrument); mercury manometer; respiration tambour (Marey's form); signal pen to record stimulations, injections and other events; time signal; stimulating coil and accessories complete; and a jacketed burette for transfusing warm solutions into the vein. The recording pens of the manometer, tambour, signals, etc., must all be adjusted to the kymograph in an exact vertical line. Fill the lead tube of the manometer with 10 percent magnesium sulphate from a pressure bottle, take the zero level of the manometer, set the time signal to write on this level, connect with the cannula and fill to a pressure of 130mm mercury. Connect the respiration tambour directly with the side branch of the tracheal T-tube. Start the kymograph, ink all the pens, remove the arterial bull-dog clamp and the experiment is ready to begin.

A renal onkometer record should often be taken with blood pressure. Open the abdomen along the entire median

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line, cut the wall transversely for 2 to 3 inches over the left kidney. Strip the kidney of its fat and looser coverings and enclose in a renal onkometer. Adjust the onkometer overflow to exact kidney level and take a record of the variations with a small sized Brodie's bellows recorder adjusted in line with the recorders mentioned above.

METHOD OF TESTING THE ACTION OF DRUGS ON THE REFLEXES OF A FROG.

Carefully destroy the brain above the medulla. Prevent the loss of blood. Suspend the frog to a horizontal rod on a stand using a card hanger or a loop of string on the upper jaw. Stimulate the tip of the toe with acid or with platinum electrodes and measure the reaction time by counting seconds until the foot is withdrawn. The reaction time may be recorded on a kymograph. Attach a horizontal writing point of mucilaged paper to the leg above the foot. Take the speed of the drum with one magnet beating seconds and record the instant of stimulation with a second and independent magnet.

Take the normal reaction time first, then give the drug as an injection in the dorsal lymph sac and allow about 20 minutes for absorption. Remeasure the reaction time and repeat at intervals of 10 minutes to get the progressive effects of the drug.

METHOD OF GIVING AND TESTING THE ACTION OF A DRUG ON THE FROG'S GASTROCNEMIUS MUSCLE.

One should always compare the drugged muscle with a normal or undrugged muscle from the same frog. Lay a ligature about one leg near the thigh tight enough to stop the circulation. Give the drug, usually by injection into the dorsal lymph sac, and after absorption is complete and the tissues have been acted on by the drug, dissect out the gastrocnemii and test them. Always use the drugged muscle first and the normal immediately following. The muscles may be removed and mounted in a moist

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chamber, or the frog may be pinned to a platform and the tendons exposed and attached to a muscle lever in the usual horizontal position. It is best to stimulate the muscle directly. There are three tests that can be applied; 1, Irritability, by the minimal stimulus method; 2, Rapidity of the simple muscle contraction; 3, The amount of work a muscle will do with simple contractions at constantly repeated intervals. In this latter test stimulate once in two seconds, record on a drum with speed of 1 mm per second.

IRRIGATING FLASKS.

The quarter and half pint aspirator bottles manufactured by Whitall, Tatum & Co., with tubed foot for attaching a rubber tube are particularly adapted to both irrigation and perfusion of the heart. For use in irrigation these bottles are clamped to a heavy base stand by a universal burette clamp on the neck. Insert a tight fitting rubber stopper with a 2mm glass tube to give constant pressure level. A short heavy rubber connector provided with a small screw compress and a glass dropper serves to regulate the speed of the outflow. Such a flask attched to an independent stand and set at a level so that the fluid drops fall only a few millimeters is an exceptionally satisfactory method of applying solutions directly to the heart.

Two aspirator bottles may be connected together by a T-tube for perfusion work. In this case the inflow cannula is connected by a very short (6 to 10 cm) tube of small calibre and is supported firmly by a clamp on the T-tube. The connecting tubes for the flasks are 25 to 30 cm long to permit adjusting. Set screw compresses near the T-tube. Fill one flask with the normal solution, the other with the drug. A very small amount of fluid can be applied by means of these perfusion flasks.

TRANSFUSION BURETTE FOR MAMMALS.

Transfusions of several cubic centimeters of liquid should be warmed to body temperature. Inclose a 50cc

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burette in an ordinary Liebig's condenser jacket and mount vertically on a heavy base stand. Mount and connect a 6-inch funnel with the upper side tube of the condenser. Attach a rubber tube fitted with a screw compress clamp on the lower side tube to regulate the outflow of the warm water introduced by the funnel to keep the perfusion liquid at the proper temperature. Mount a thermometer inside the condenser with its bulb near the lower end of the apparatus. The burette connections with the transfusion cannula should be as short as possible. Where only 1 or 2 cc of liquid is to be introduced it is unnecessary to warm it. In fact a hypodermic syringe is most convenient where the volume of the injection does not exceed 1.5cc.

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LIST OF SOLUTIONS.

Aconite 0.1 percent.
Adrenalin hydrochloride 0.001 percent, 0.01 percent, 0.05 percent 0.1 percent.
Alcohol 95 percent; 2 percent, 5 percent, 10 percent, 20 percent.
Amyl nitrite 0.1 percent, pearls.
Atropine 0.001 percent, 0.002 percent, 0.1 percent, 0.2 percent, 1 percent, 2 percent, 5 percent, and 1-120 grain tablet.
Barium chloride 0.01 percent, 0.1 percent, 0.2 percent, 1 percent.
Caffeine 0.1 percent, 0.2 percent, 0.5 percent, 1 percent.
Calcium chloride 0.03 percent, 0.06 percent, 1 percent.
Carbolic acid 0.5 percent, 1 percent, 10 percent.
Chloral hydrate 1 percent, 2 percent.
Chloroform 0.05 percent, 0.1 percent, 0.5 percent; 20 percent in oil; pure.
Cocaine hydrochlorate 0.01 percent, 0.2 percent, 0.5 percent, 1 percent, 2 percent.
Codeine 0.5 percent, 1 percent.
Curara 0.2 percent, 1 percent.
Digitalis 0.0005 percent, 0.001 percent, 0.002 percent, 0.1 percent, 0.2 percent, 0.5 percent, 1 percent.
Ether 1 percent, 2 percent, 4 percent, 6 percent, 8 percent, pure.
Ergot Squib's fluid extract, 10 percent of fluid extract.
Hyoscyamine 1 percent.
Nicotine 0.02 percent, 0.1 percent, 0 2 percent, 1 percent.
Nitroglycerine 0.1 percent.
Physiological saline 0.7 percent.
Physostigmine 0.1 percent, 1 percent.
Pilocarpine nitrate 0.1 percent, 1 percent, 10 percent.
Potassium chloride 0.03 percent, 1 percent, 5 percent.
Potassium bromide 5 percent.
Quinine hydrochlorate 0.1 percent, 1 percent.
Ringer's solution, weak, strong.
Sodium nitrite 0.01 percent, 0.02 percent.
Sodium sulphate 1 percent.
Thebaine 0.5 percent, 1 percent.
Veratrine 0.05 percent, 0.1 percent, 1 percent; 1 percent of fluid extract.

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